IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE Application of >
United States Serial No. 10/740,264 filed
7 July 2000
FOR "QUINUCLIDINE DERIVATIVES AND THEIR USE AS MUSCARINIC M3 RECEPTOR LIGANDS"

DECLARATION

I, Amadeu Gavaldà, a Spanish citizen of Cardener 68-74, 08024 Barcelona , Spain, BSc PhD, current position: Biological Programme Leader since 1997 at Almirall and Pharmacologist since 1990 at Prodesfarma.

hereby declare:-

1. I am familiar with the Spanish and English languages.

2. The following tests were carried out under my direct supervision to determine the biological activity of the compounds described and claimed in the above-identified application:

(a) Human muscarinic receptor studies

The binding of [^{3}H]-NMS to human muscarinic receptors was performed according to the procedure described by M. Waelbroek, M. Tastenoy, J. Camus and J. Christophe; Binding of selective antagonists to four muscarinic receptors (M1 to M4) in rat forebrain;

Mol. Pharmacol, (1990) 38: 267-273. Assays were carried out at 25°C. Membrane preparations from stably transfected chinese hamster ovary-K1 cells (CHO) expressing the genes for the human muscarinic receptors Hm3 were used.

For determination of IC_{50}, membrane preparations were suspended in DPBS to a final concentration of 89μg/ml for the Hm3 subtype. The membrane suspension was incubated with the tritiated compound for 60 min. After incubation the membrane fraction was separated by filtration and the bound radioactivity determined. Non specific binding was determined by addition of 10^{-4} M atropine. At least six concentrations were assayed in duplicate to generate individual displacement curves.

The following results were obtained

COMPOUNDS No	BINDING TO RECEPTOR M_3 (IC_{50} nM)
ATROPINE	3.2
IPRATROPIUM	3.0
99	31
100	14
101	7.6
109	31
114	14
116	23
126	13
127	16
128	8.8
129	6.3
136	11

137	6.9
138	19
146	13

The results obtained show that the compounds of the above-identified application have affinities for the M_3 receptors which are very similar to the reference compounds.

(b) Test on bronchospasm in guinea pig

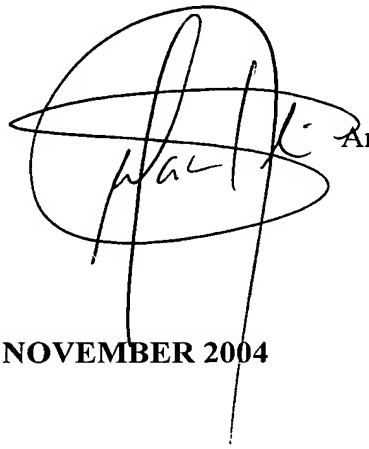
The studies were performed according to the procedure described by Konzett H., Rössler F., Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. Arch. Exp. Path. Pharmacol. 195: 71-74 (1940). Aqueous solutions of the compounds to be tested were nebulised and inhaled by anaethetised ventilated male guinea pigs (Dunkin-Hartley). The bronchial response to intravenous acetylcholine challenge and the percentage change in pulmonary resistance at several time-points were determined before and after administration of the compound under test.

The tested compounds inhibited the bronchospasm response to acetylcholine with high potency and a long duration of action.

4. The activities of the compounds subjected to the test procedures described above demonstrate the antimuscarinic activity (M_3) of the compounds described and claimed in USSN 10/740264. Such antimuscarinic activity is known to be associated with utility in the treatment of respiratory, urinary or gastrointestinal diseases in which the muscarinic M_3 receptor is implicated. The measured affinity levels for human muscarinic M_3 receptors (Hm3) are very similar to those of the reference compounds atropine and Ipratropium.

5. The undersigned declares further that all statements made herein of his/her own knowledge are true and that all statements made on information and belief are believed to be true; further that these statements are made with the knowledge that

wilful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the Application or any Patent issuing thereon



Amadeu Gavaldà PhD

DATED THIS 19 DAY OF NOVEMBER 2004

Selective muscarinic agonists and antagonists provide an advance over earlier, nonselective therapeutics where the clinical efficacy was compromised by the side effect profile. In terms of basic research, these and other compounds will be important tools to pharmacologically characterize muscarinic receptor subtypes.

Muscarinic Receptor Subtypes: Pharmacology and Therapeutic Potential

by Richard M. Eglen and Sharath S. Hegde

MUSCARINIC RECEPTORS MEDIATE the cellular actions of acetylcholine at the parasympathetic neuroeffector junction. In addition, these receptors mediate cholinergically mediated effects in the central nervous system (CNS), particularly in cortical and subcortical regions of the brain.[1] In both the CNS and periphery, muscarinic receptors are located pre- or postjunctionally and mediate both excitatory and inhibitory effects.[1] It was anticipated, therefore, that these effects involved more than a singular subtype.

Classically, muscarinic receptors are defined by selective agonism with muscarine and antagonism with atropine, respectively.[2,3] Early pharmacological studies with the muscarinic receptor antagonists 4-DAMP[4] or gallamine[5] indicated that muscarinic receptors existed in at least two subtypes, with those mediating smooth muscle contraction differing from those mediating negative inotropy. Subsequently, radioligand binding studies with the antagonist pirenzepine identified muscarinic receptor heterogeneity in cerebral cortex, salivary gland and stomach fundus.[6]

However, the true extent of muscarinic receptor heterogeneity has come from purification[7] and, subsequently, cloning techniques[8] which revealed the existence of five muscarinic receptor subtypes, encoded by distinct genes. Although highly homologous, these receptors differ in their primary sequence, intracellular effector systems and tissue distribution.[9]

Pharmacologically, differentiation among these *five* subtypes, even those expressed in recombinant expression systems, is complex, since few ligands are currently available with marked selectivity for a single subtype.[10] Consequently, operational identification of a muscarinic receptor relies upon the affinity profile of several antagonists.[11] This situation, exacerbated in cells expressing multiple receptors, is complicated by an absence of selective muscarinic receptor agonists.[12] Collectively, delineation of the physiological role of each subtype remains difficult.

A major goal of muscarinic receptor research, therefore, is to identify subtype-selective agonists and antagonists. These agents will provide important tools in assigning a physiological role to each muscarinic receptor subtype. Therapeutically, such compounds will exhibit fewer clinical side effects than those classically associated with nonselective muscarinic agonists and antagonists. Consequently, several compounds are now in advanced clinical evaluation for a variety of disorders, ranging from cognitive dysfunction to urinary incontinence.

This article will discuss selective muscarinic receptor agonists and antagonists (see Tables I-III), both in the context of novel therapeutics and as agents to define receptor subtypes. An attempt has been made to ensure that this overview is current. Consequently, several references are from papers either in press or abstracts recently published. Additional aspects of muscarinic receptor research can be found in several recent reviews.[12–15]

Characterization of muscarinic receptor subtypes

Muscarinic receptor genes encode five distinct receptor proteins, denoted m_1, m_2, m_3, m_4 and m_5 subtypes, that conform to the archetypal G-protein–coupled receptor motif (Table I). The cloning and expression of these cDNAs encoding muscarinic receptors has facilitated studies into the function and regulation of these subtypes at a molecular level. In addition, the widespread availability of these clones, including those of human origin, has simplified the determination of the pharmacological profile for a single muscarinic receptor subtype since ligand affinities can be determined at a single recombinant receptor. Indeed, the antagonist affinities at cloned receptors, particularly those derived from studies conducted in buffers of physiological ionic strength,[16] concur with those generated at endogenously expressed receptors (Table I).

In the future, data will undoubtedly emerge from studies in transgenic animals lacking genes for each of the subtypes, thus allowing insights into their functional role. Until then, implicating a muscarinic receptor in a tissue response is undertaken by measuring the affinities of several antagonists (Fig. 1). Due to the poor discrimination of the compounds between these receptors, and the propensity of ligands to act allosterically, the optimal use of these compounds mandates the measurement of affinities under conditions of equilibrium.[17] Pharmacological criteria currently define four muscarinic receptor subtypes, denoted muscarinic M_1, M_2, M_3 and M_4 receptors (Table I). Antagonists useful in classification include atropine (nonselective), pirenzepine (M_1 selective), tripitramine and methoctramine (M_2 selective), himbacine (M_2/M_4 selective), 4-DAMP (M_1/M_3 selective), p-F-HHSiD and, most recently, darifenacin (M_3 selective).[18]

The muscarinic M_4 and m_5 receptors have hitherto been difficult to characterize due to a lack of selective ligands and limited tissue distribution. In the case of the former, a novel antagonist has been described, PD-102807,[19] that may aid characterization. Moreover, MT-3, a compound isolated from a snake toxin, is perhaps the most selective and reversible ligand for the M_4 or, indeed, any muscarinic receptor subtype.[20]

Currently, a physiological role for the muscarinic m_5 receptor is not clear and, to differentiate its status from that of the remaining four, it is correctly cited with the lower-case nomenclature. This subtype, for which no selective ligands have yet been identified, is generally considered to reside exclusively within the CNS.[21] Recent data, however, indicate expression of the receptor in human melanoma cells,[22] ciliary muscle[23] and mononuclear blood cells,[24] the function of which is unknown.

"Selective" muscarinic receptor agonists

Several agonists have been identified that exhibit functional selectivity for muscarinic M_1 receptors. These compounds are so termed since they preferentially activate one muscarinic receptor subtype by virtue of the prevailing high receptor reserve, as opposed to any differential affinity. Selective agonism, therefore, by novel compounds may not occur *in vivo*, since the number

TABLE I: CHARACTERISTICS OF MUSCARINIC RECEPTOR SUBTYPES[a]

Nomenclature[b]	M_1	M_2	M_3	M_4
Receptor gene	m_1	m_2	m_3	m_4
Structure	7 TM	7 TM	7 TM	7 TM
Human	460 aa	466 aa	590 aa	479 aa
Mouse	460 aa	–	–	479 aa
Rat	460 aa	466 aa	589 aa	478 aa
Porcine	460 aa	466 aa	590 aa	479 aa
Intracellular messenger	IP_3/DG	cAMP/k^+ channels	IP_3/DG	cAMP
Pharmacology[c]				
4-DAMP	8.6 (9.2)	7.8 (8.1)	9.1 (9.3)	ND (8.4)
Darifenacin	7.9 (7.8)	6.9 (7.0)	9.4 (8.9)	ND (7.7)
Himbacine	7.2 (6.6)	8.5 (7.9)	7.6 (6.9)	8.8 (7.4)
Methoctramine	6.5 (6.6)	7.9 (7.6)	6.0 (6.1)	7.6 (6.9)
p-F-HHSiD	7.2 (7.3)	6.0 (6.6)	7.9 (7.5)	ND (7.2)
PD-102807	< 5.7	5.8	6.1	7.1*
Pirenzepine	8.3 (8.0)	6.8 (6.3)	6.9 (6.9)	7.7 (7.0)
Tripitramine	ND (8.4)	9.7 (9.4)	6.5 (7.1)	ND (7.8)

[a]For a review, see reference 49.
[b]A fifth gene, m5, has been cloned, but no functional correlate has been unambiguously demonstrated.
[c]The values are affinities determined functionally. Those in parentheses are determined in radioligand binding studies at cloned human muscarinic receptors, expressed in CHO cells.
TM, predicted number of transmembrane spanning domains; aa, amino acid residues; IP_3, inositol-(1,4,5)-trisphosphate; DG, 1,2-diacylglycerol (mobilization); cAMP, 3′,5′-cyclic adenosine monophosphate (inhibition); *, relaxation of rabbit anococcygeus muscle [75]; ND, not determined.

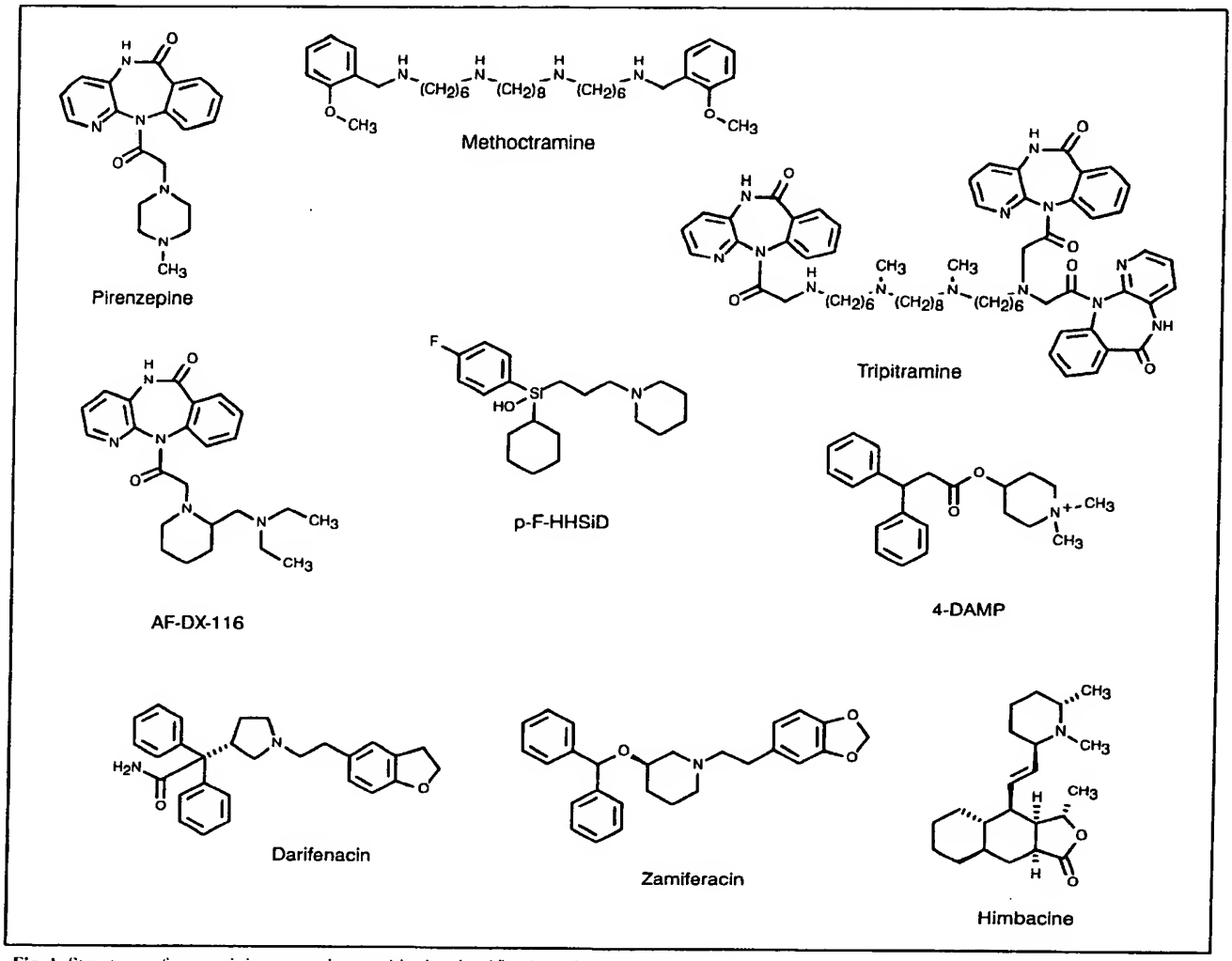


Fig. 1. Structures of muscarinic antagonists used in the classification of receptor subtypes.

of receptors, G-proteins or both may differ from that expressed in isolated cells and tissues. A potential complicating factor is the effect of disease pathology on receptor number and/or efficiency of signal transduction.

Alzheimer's disease

Alzheimer's disease is a condition associated with an accelerated decline in cognitive function, causally related to a deterioration of cortical cholinergic neurons.[25,26] Since postjunctional muscarinic M_1 receptors are preserved as the disease progresses, selective activation of muscarinic M_1 receptors may provide a better approach to treatment of this disorder. The therapeutic value of selective M_1 receptor agonists in the treatment of Alzheimer's disease is clinically unproven, but they clearly have potential, given the clinical utility of acetylcholinesterase inhibitors in this disease. Furthermore, preclinical studies suggest that muscarinic M_1 receptor activation, by agonists such as xanomeline and AF-102B, modulates amyloid precursor protein processing.[27] Collectively, these data indicate that selective muscarinic M_1 receptor agonism could exert both a palliative and ameliorative approach to the disease.

Many muscarinic receptor agonists are under advanced development (Table II) including muscarones, spirodioxolanes, derivatives of RS-86, pilocarpine, oxotremorine and arecoline.[28] Arecoline, in particular, has been extensively substituted such that some oxadiazole derivatives are some of the most potent and efficacious muscarinic receptor agonists identified to date.[28] A feature of several agonists of this class is that they exhibit only slight to moderate side effects in healthy volunteers, as well as reversing cognitive dysfunction in animal models.

AF-102B, for example, is well tolerated at doses up to and including 50 mg orally.[29] Milameline, also an arecoline analogue, is functionally selective for M_1 over M_2 receptors.[30,31] Phase I safety studies show minimal side effects[31] of this agonist at doses of 4–8 mg/day in the elderly.

TABLE II: FUNCTIONALLY SELECTIVE MUSCARINIC M_1 RECEPTOR AGONISTS IN PRECLINICAL OR CLINICAL DEVELOPMENT

COMPOUND	PHASE	COMPANY/INSTITUTION
COGNITIVE DYSFUNCTION		
AF-102B	Phase III	Snow Brand
AF-150S	Preclinical	Israel Inst. Biol. Res.
AF-185	Preclinical	Israel Inst. Biol.Res.
CDD-0199-J	Preclinical	University of Toledo
HP-184	Phase II	Hoechst Marion Roussel
KST-2818	Preclinical	Kaneka
KST-5410	Preclinical	Kaneka
L-689660	Preclinical	Merck & Co.
L-705106	Preclinical	Merck & Co.
Lu-25109	Phase I	Lundbeck
LY 287041	Phase I	Eli Lilly
MDL-74019	Preclinical	Hoechst Marion Roussel
Milameline	Phase III	Warner-Lambert/Hoechst Roussel
PD-141606	Preclinical	Warner-Lambert/Parke-Davis
PD-142505	Preclinical	Warner-Lambert/Parke-Davis
PD-151832	Preclinical	Warner-Lambert/Parke-Davis
PDC-008004	Preclinical	Pharmaceutical Discovery
RU-35963	Preclinical	Hoechst Marion Roussel
S-9977-2	Phase II	Servier
SB-202026	Phase III	SmithKline Beecham
SR-46559A	Phase II	Sanofi
SDZ-210086	Preclinical	Novartis
Talsaclidine	Phase II	Boehringer Ingelheim
U-77053	Preclinical	Pharmacia & Upjohn
U-80816	Preclinical	Pharmacia & Upjohn
Xanomeline	Phase III	Eli Lilly
YM-796	Phase II	Yamanouchi
YM-954	Preclinical	Yamanouchi
GLAUCOMA		
DD-22A	Preclinical	Leiras/Huhtamaki
L-696986	Preclinical	Merck & Co.
PAIN DISORDERS		
LY 297802	Phase II	Eli Lilly/Novo Nordisk

SR-46559A, a derivative of the antidepressant minaprine,[32] and SB-202026[33] are in phase II and III clinical trials, respectively, while other agonists are at a similar or later stage of development. These include talsaclidine,[34] which at doses of 60 mg orally and less is devoid of significant side effects, although at doses of 40–160 mg, cholinergic side effects were apparent.[34] YM-796 is also in a phase II clinical trial in Japan for Alzheimer's disease, although no clinical data are yet available. Xanomeline,[35] in a phase I study, exhibited no significant side effects at 75 mg orally, although they are seen at 100 and 150 mg, with the maximal tolerated dose being 300 mg/day. In a 343-patient double-blind placebo-controlled trial, the compound improved cognition and other symptoms, as well as increased score on a primary care-giver scale.[36] To improve control over blood levels a transdermal patch is now under development.

Pain disorders

Central administration of muscarinic agonists has been shown to evoke analgesic effects in several animal models.[37] although the subtype(s) of muscarinic receptor underlying the antinociceptive response has not been elucidated. Emerging preclinical data suggest a spinal site for the locus of action, via a pertussis toxin-sensitive G-protein.[38] Although this receptor remains to be clarified, these data may implicate either muscarinic M_2 or M_4 receptors in the response. Nonselective partial agonists, such as LY-297802, are in development for the treatment of pain disorders.[39] To date no clinical efficacy has been reported.

Glaucoma

Muscarinic agonists can facilitate drainage of aqueous humor in glaucoma via contraction of ciliary muscles.[40] Pilocarpine has been shown to be useful in the treatment of chronic glaucoma,[41] and a number of compounds have also been synthesized for use in this condition, including DD-22A, which acts as an ester prodrug of pilocarpine,[42] and L-696986, a mixed muscarinic M_1/M_3 agonist/M_2 antagonist that reduces intraocular pressure in primates.[43]

Selective muscarinic receptor antagonists

Antagonism of muscarinic receptors is an attractive therapeutic strategy inasmuch as the cholinergic system has been implicated in several disorders including peptic ulcer, asthma, chronic obstructive pulmonary disease, irritable bowel syndrome and urge incontinence. Classic muscarinic receptor antagonists, such as atropine, do not distinguish between muscarinic receptor subtypes. This limits their therapeutic utility owing to the occurrence of side effects such as mydriasis, xerostomia, CNS disturbances, tachycardia and constipation.[44] Table III lists the characteristics of some muscarinic receptor antagonists under development.

Peptic ulcer disease

Pirenzepine, an antagonist with relatively high affinity for the muscarinic M_1 and modest affinity for the musca-

TABLE III: SOME MUSCARINIC RECEPTOR ANTAGONISTS UNDER DEVELOPMENT

COMPOUND	PHASE[a]	RECEPTOR SELECTIVITY[b]	INDICATION	COMPANY
ALIMENTARY TRACT				
Darifenacin	Phase III	M_3	Irritable bowel syndrome	Pfizer
Telenzepine	Preregistered	M_1/M_4	Antiulcer	Byk Gulden
ANTIARRHYTHMIC				
Otenzepad	Phase II	M_2/M_4	Bradycardia	Boehringer Ingelheim
Ebeinone	Preclinical	M_2/M_4	Bradycardia	University of Karachi
GENITOURINARY				
Darifenacin	Phase III	M_3	Urinary incontinence	Pfizer
NS-21	Phase II	NS	Urinary incontinence	Nippon Shinyaku
Tolterodine	Registered	NS	Urinary incontinence	Pharmacia & Upjohn
Vamicamide	Preregistered	NS	Urinary incontinence	Fujisawa
YM-46303	Phase I	M_3	Urinary incontinence	Yamanouchi
RESPIRATORY				
Tiotropium*	Phase II	NS	Antiasthma	Boehringer Ingelheim
Rispenzepine	Discontinued	M_3/M_1	Antibronchospastic	Dompe

[a]Preregistered, marketing application submitted; Registered, marketing application approved. [b]NS, nonselective. *, preferential slow off-rate from the muscarinic M_3 receptor.

rinic M_4 receptor, is approved for clinical use in the treatment of peptic ulcer disease.[45] A structurally related compound in advanced clinical development is telenzepine,[46] which exhibits a longer duration of action that permits once-daily dosing and comparable efficacy in the treatment of peptic ulcer remission. In a double-blind comparative trial with ranitidine, telenzepine possessed comparable efficacy when ulcer rates were measured, although other symptoms improved more rapidly with ranitidine.[47] It is also arguable, but not proven, that a highly selective muscarinic M_3 receptor antagonist may be useful in the treatment of peptic ulcer disease, given the role of this subtype in regulating parietal cell secretion.

Smooth muscle disorders (asthma, chronic obstructive pulmonary disease, irritable bowel syndrome, urge incontinence)

An established indication for muscarinic receptor antagonists is to relax smooth muscle, with the degree of relaxation produced depending upon the level of prevailing parasympathetic nervous tone. Muscarinic receptors are intimately involved in controlling smooth muscle function. Physiologically, muscarinic M_1 receptors are present on parasympathetic ganglia, located close to the effector organ, where they serve to modulate cholinergic transmission. At the end-organ terminals, the release of acetylcholine is modulated, usually in an inhibitory fashion, by a prejunctional M_2, M_3 or M_4 muscarinic autoreceptor.[48]

Contractile responses of smooth muscle to acetylcholine are mediated by activation of postjunctional muscarinic receptors, the nature of which varies according to species and anatomical location. In many, although not all, smooth muscles studied, including those of human origin, muscarinic M_3 receptors mediate contraction. Surprisingly, postjunctional muscarinic M_3 receptors are present in low numbers (25% or less), with most smooth muscles possessing a preponderant muscarinic M_2 receptor population.[49,50]

Since muscarinic M_2 receptors inhibit β-adrenoceptor–stimulated adenylyl cyclase activity in smooth muscle,[51] a potential role for muscarinic M_2 receptors in the modulation of relaxant responses to β-adrenoceptor agonists has been suggested. In the absence of a prevailing relaxant tone, specifically that provided by activation of adenylyl cyclase, muscarinic M_2 receptors appear to play no role. In contrast, under conditions of high sympathetic activity (and thus adenylyl cyclase activity is elevated) or where muscarinic M_3 receptors are dysfunctional, M_2 receptors could provide the dominant parasympathetic control over gastrointestinal smooth muscle tone.[52]

This aspect of muscarinic receptor function in human smooth muscle physiology is not well explored and, indeed, the potential for pathophysiological changes in the muscarinic M_2:M_3 ratio is not known. Nonetheless, it has been suggested that the development of antagonists with affinity for both muscarinic M_2 and M_3 receptors could provide optimal inhibitory control of smooth muscle contraction.[49] It is unknown, however, if the disease pathology influences the roles of these two subtypes in smooth muscle hypermotility.

Given the effectiveness of nonselective muscarinic receptor antagonists, selective blockade of muscarinic M_3 receptors in smooth muscle will confer a therapeutic advantage in the treatment of respiratory disorders, such as chronic obstructive airway disease or asthma, in gastrointestinal disorders, such as irritable bowel syndrome, and in urinary tract disorders, such as urge incontinence.

Vagal stimulation induces bronchoconstriction and mucus secretion, by activation of muscarinic receptors located on smooth muscle, vascular endothelium, submucosal cells and neural elements.[53] Since cholinergic neural mechanisms may contribute to airway narrowing in asthma and chronic obstructive airway disease, muscarinic receptor antagonists are effective in treating acute bronchoconstriction, particularly that occurring in chronic obstructive airway disease.[54] Antagonists currently available for the treatment of this condition are nonselective and exhibit the side effects discussed above. Novel antagonists such as LG-50643[55] and NPC-14695[56] exhibit selectivity toward airway muscarinic receptors, although clinical data are not available. Rispenzepine (DF-594), in contrast, was in phase II/III clinical trials as an antibronchospastic,[57] although development is now discontinued.

Some therapeutic approaches to selective blockade of airway smooth muscle exploit differences in receptor kinetics or absorption. Ipratropium is a quaternized derivative of atropine that is poorly absorbed into the systemic circulation when given by inhalation.[58] Although nonselective between subtypes, the poor absorption following inhalation facilitates selective antagonism of airway muscarinic receptors. Tiotropium bromide[59] is an antagonist with a preferential slow off-rate from muscarinic M_3 receptors with respect to muscarinic M_2 receptors. This compound is currently in phase II clinical trials, both as an antiasthmatic agent and for chronic obstructive pulmonary disease. The prolonged duration of action suggests it may also have potential in the treatment of nocturnal asthma.

The parasympathetic nervous system has been implicated in abnormal motility patterns associated with irritable bowel syndrome. Nonselective muscarinic antagonists such as cimetropium and octylinium have been used in the treatment of irritable bowel syndrome, although the efficacy of these compounds is questionable.[60] Several relatively old compounds, including dicyclomine, pinaverium, fendoverine, mebeverine and milverine, have also been used for slowing gut motility. Although lacking selectivity for M_3 receptors, they possess other properties, including calcium channel blockade, a property that also contributes to their antispasmodic effects.[61] Newer compounds with selectivity for M_3 receptors, including zamifenacin[62] (the development of which is now discontinued) and darifenacin,[63] have been developed which show apparent gut selectivity in animal models.

Muscarinic antagonists are frontline agents in the pharmacotherapy of urge incontinence associated with detrusor hyperactivity since the parasympathetic nervous system represents the principal excitatory drive to the urinary bladder.[64] Currently, oxybutynin and propantheline are the two most commonly used compounds for this purpose. Among the compounds that are being clinically evaluated, darifenacin[65] and tolterodine[66] are in the most advanced stages of development. While darifenacin displays selectivity for M_3 receptors,[65] tolterodine has equal affinity for all the five subtypes.[66] Darifenacin is expected to cause less tachycardia, compared to tolterodine, owing to its low affinity for M_2 receptors. Both compounds have been claimed to possess marginal selectivity for the bladder over salivary gland and reported to cause a slightly lower incidence of dry mouth in clinical trials.[67,68] The mechanistic basis for this observation is unclear, and it would be important to define the relative efficacy of the two compounds in reducing detrusor hyperactivity, given that both M_2 and M_3 receptors may be of functional importance in the bladder.[69] Vamicamide,[70] NS-21[71] and YM-46303[72] are muscarinic antagonists which possess negligible or modest selectivity for M_3 receptors and are in different stages of clinical development for urge incontinence. NS-21 is distinct from the other two compounds in that it is also a potent calcium channel blocker.

Cardiac arrhythmias

Certain bradycardic disorders are associated with exaggerated vagal drive to the heart. Since M_2 receptors mediate the cardiac effects of acetylcholine, selective M_2 receptor antagonists, such as otenzepad (AF-DX-116), may be useful in the treatment of sinus bradycardia[73] and would be devoid of anticholinergic side effects such as dry mouth and constipation. Otenzepad is currently in phase II clinical trials in Germany and Japan.

Parkinson's disease

In situ hybridization and antibody studies have shown that M_4 receptors dominate in striatal regions of the brain,[74] where they may modulate dopaminergic neurotransmission, either via postsynaptic mechanisms or by regulating neurotransmitter release through inhibitory heteroreceptors. Accordingly, selective muscarinic M_4 antagonists, such as analogs of PD-102807,[19] have been developed which may exert a beneficial action in Parkinson's disease.

Conclusions

It is now established that muscarinic receptors exist in multiple subtypes. Collectively, research into muscarinic receptor subtypes is advancing at a rapid pace, both fundamentally and clinically. Particularly noteworthy is the emergence of more selective ligands, as both research tools and novel therapeutics. Pharmacologically, the characterization of muscarinic receptors remains difficult, though not impossible. While the available functionally selective muscarinic agonists do not provide an unambiguous means for receptor characterization, they have potential as useful therapeutics in the treatment of Alzheimer's disease. At present, it is unknown if the effects will be seen in only a subset of the patients and whether

the efficacy will be equal to or greater than that seen with some of the newer cholinesterase inhibitors. Muscarinic receptor antagonists are clearly useful in defining muscarinic receptor subtypes, and in at least three areas of smooth muscle pathology, selective muscarinic M_3 receptor antagonism may be of therapeutic benefit. The approval of compounds for the treatment of respiratory and urological disorders will probably occur in the next few years and may prove an advance over existing therapies.

Taken together, the recognition of multiple muscarinic receptor subtypes is accelerating the design of novel drugs for a variety of diseases. The approval of these compounds in the next five years or so will enable their potential to be assessed as novel therapeutics. Moreover, given the widespread role of acetylcholine as a central and peripheral neurotransmitter, future research will undoubtedly disclose additional applications for subtype selective ligands.

Acknowledgments

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